Applicant: JORSBOE ET AL.
Serial No.: 09/762,629
Amendment dated June 10, 2003
Reply to Office Action Feb. 12, 2003

REMARKS

Pending claims 74-89 have been re-numbered to correct a typographical error in the previous Amendment wherein now corrected Claim 76 had been mistakenly labeled as a second Claim 75, therefore previous Claims 76 - 89 are now re-numbered as Claims 77- 90. Throughout these Remarks, the claims are referred to by their corrected numbering after entry of the present amendment. Claims 74-77 and 81-83 have been amended to clarify Applicants' invention. No new matter is added by the amendments. Support for the amendment to claim 74 can be found, for example, at page 60, lines 6-7 of the application ("it is desirable to use up to 3 genes encoding enzymes useful for the conversion of galactose to UDP-glucose"). Support for the amendments to claims 81-83 can be found, for example, at page 46, line 12 to page 47, line 5 ("[t]his D-galactose can be supplied to the plant cells or tissues during selection as free D-galactose or as a D-galactose containing compound"). Applicants request reconsideration of the outstanding objections and rejections in this application.

Written description

Claims 74-90 stand rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventor had possession of the claimed invention.

The Examiner states that, though Applicants claim polynucleotides encoding enzymes that enhance conversion of galatactose to UDP-glucose, Applicants do not adequately describe the nucleotides or methods of their use.

Applicants respectfully traverse this rejection. The standard for determining whether an application complies with the written description requirements of § 112, first paragraph, is

whether one of ordinary skill in the art recognizes from reading the disclosure, that the inventors were in possession of the claimed subject matter as of the filing date. Additionally, "a description as filed is presumed to be adequate, unless or until sufficient evidence or reasoning to the contrary has been presented by the Examiner to rebut the presumption." MPEP 2163.04.

Applicants respectfully submit that, in view of the foregoing guidelines, the disclosure clearly provides adequate written description for the invention as claimed. The pending claims recite processes for selecting plant cells or tissue that are transformed with one or more polynucleotides encoding enzymes useful to convert galactose to UDP-glucose. Applicants submit that enzymes of the galactose metabolic pathway that results in conversion of galactose to UDP-glucose are well known to those of skill in the art. See, e.g., *Biochemistry*, (Voet & Voet, Second Edition, John Wiley & Sons, Inc., Somerset, N.J. 1995, p. 477-478) (copy attached). Further, polynucleotides encoding these enzymes are also well known in the art, and are publicly available, for example, via online databases.

Four enzymes in the galactose metabolic pathway are disclosed as useful in the invention: UTP-dependent pyrophosphorylase (EC 2.7.7.10), UDP-glucose-dependent uridyl transferase (EC 2.7.7.12), galactokinase (EC 2.7.1.6), and UDP-galactose epimerase (EC 5.1.3.2) (page 60). Therefore, the disclosure provides the official Enzyme Commission classification numbers for these enzymes, thereby allowing one of skill in the art to immediately ascertain the identity of these enzymes, as well as obtain significant information about their characteristics through readily available online databases. These databases provide general information for these enzymes, as well as links to their amino acid sequences and sequences of polynucleotides encoding these enzymes from a wide variety of species. Applicants exemplify the

transformation of plant cells with a *galT* gene obtained from *E. coli*. Attached are sequences from online databases for each of the four above-named enzymes obtained from *E. coli*.

Because the polynucleotides within the scope of the claims are well known and readily defined and accessible via the EC numbers provided in the specification, Applicants assert the polynucleotides recited in the method claims are adequately described. Applicants submit, therefore, that the Examiner has failed to rebut the presumption of adequate description as required, at least for this reason. (MPEP 2163.04). Withdrawal of this rejection is respectfully requested.

Enablement

Claims 74-90 stand rejected under 35 U.S.C. § 112, first paragraph. The Examiner asserts the specification, while enabling the transformation of potato and oil seed rape cells and tissue using the *E. coli galT* gene and selection for transformed material, allegedly does not enable the transformation of all types of cells and tissues. The Examiner further asserts that the screening for plants that escape the selection process is unpredictable and requires further experimentation to determine the best conditions of selection for each and every variety or species claimed.

Applicants respectfully traverse this rejection. In order to make an enablement rejection, "the examiner has the initial burden to establish a reasonable basis to question the enablement provided for the claimed invention." MPEP 2164.04.

Furthermore, whether the claims are enabled must be determined from the point of view of the level of skill and knowledge in the art. Applicants submit that one of skill in this art is a person having considerable training and at least an undergraduate degree and often an advanced degree. One of skill in this art has special expertise in working with plant cells or tissue, and has considerable knowledge of techniques for transformation of plant cells or tissue, plant cell

culturing and tissue growth, and screening procedures for selecting transformed plant cells or tissue. In this context, it is significant that Applicants' claims are limited to plant cells or tissue.

Applicants' claims are directed to a process for selecting transformed plant cells or tissue that are insensitive to galactose toxicity, wherein galactose is toxic to nontransformed cells or tissue. Applicants have disclosed that most plant species are unable to sustain growth with galactose as the carbon source (page 44, lines 17-19). Applicants have further demonstrated that galactose is toxic to a wide variety of plant species, including wheat, sunflower, oil seed rape, potato, sugar beet, and peas (Example 3).

Furthermore, Applicants have disclosed multiple working examples that exemplify the transformation of plant cells followed by selection using the methods of the invention. Applicants specifically provide details for the transformation of maize plants with a *galT* gene obtained from *E. coli*, along with details of the conditions used for the selection of the transformed maize (Example 2). Similarly, Applicants provide working examples for the transformation and selection of potato cells (Example 4) and oil seed rape (Example 5) with *galT* from *E. coli*. The disclosed working examples are notable for the diversity of species used. Maize plants, for example, are classified as monocots, while oil seed rape plants are dicots. Potato, being a tuber, is even more distinct. Thus, Applicants have provided multiple working examples of the claimed processes in plant species of widely varying biological structure and botanical classifications. The Examiner has provided no reason that the claimed invention, as exemplified by the Examples and disclosures, would not be expected to function similarly over the range of plant cells and enzymes as claimed.

The enzymes involved in the metabolism of galactose, along with the polynucleotides that encode them, are well known in the art as discussed above with respect to the written description rejection.

The determination of whether the claims are enabled, therefore, must be considered in light of the fact that 1) sensitivity to galactose exists in almost all plant species, 2) the disclosure provides guidance for the transformation of three widely varying types of plant cells with *galT* from *E. coli*, followed by selection of transformed cells that insensitive to galactose toxicity and 3) enzymes of the galactose pathway from a wide variety of species are well known and characterized, including polynucleotides encoding these enzymes. Applicants submit that in light of the foregoing, there would be no undue burden on one skilled in the art, and who possesses the specialized skills and knowledge of techniques used in plant molecular biology, to adapt the provided examples to other types of plant cells, or to other enzymes within the galactose metabolic pathway.

The Examiner asserts that rate limiting steps may occur after the introduced enzymatic activity that would negate the desired selectable advantage of the invention. The Examiner cites a reference by Dormann et al. on the role of UDP-glucose epimerase in the carbohydrate metabolism of *Arabidopsis*, and asserts that the reference shows an enzyme downstream of galactokinase and UDP-glucose uridylyltransferase in the galactose metabolic pathway that is not induced in the presence of galactose.

Applicants respectfully disagree with this aspect of the enablement rejection as well. Applicants have provided detailed examples demonstrating the successful selection of cells transformed with *galT* (encoding UDP-glucose-dependent uridyl transferase) in maize, oil seed rape, and potato cells (Examples 2, 4, and 5). This enzyme is located upstream of UDP-glucose

epimerase in the galactose metabolic pathway. Thus, Applicants have shown that transforming plant cells with an enzyme from the galactose metabolic pathway was sufficient to alleviate galactose toxicity such that it was possible to select for the transformed cells, even though the enzyme is located upstream from UDP-glucose epimerase. This showing demonstrates that no downstream rate-limiting steps negated the selective advantage of the transformed cells. Notably in this regard, many plants possess the UDP-galactose epimerase enzyme (EC 5.1.3.2), whereas it is the upstream enzymes, in particular UTP-dependent pyrophosphorylase and UDP-glucose-dependent uridyl transferase, which are typically completely absent in galactose-sensitive plants.

Additionally, Dormann et al. state that, in *Arabidopsis*, no alteration in the growth rate was observed under optimal conditions even when the activity of UDP-glucose epimerase was reduced by 90% from the wild type levels using three independent antisense lines (page 647, second full paragraph). The authors concluded from this result that "the endogenous UDP-Glc epimerase activity was present in excess in the wild-type" (page 647). Therefore, Dormann et al. produce no evidence that the selection method of the invention would fail to work even if UDP-glucose epimerase was not induced in the presence of galactose. Dormann et al. do not teach that *Arabidopsis* transformed with a polynucleotide encoding an enzyme upstream of UDP-glucose epimerase will fail to result in an insensitivity to galactose toxicity.

Furthermore, Applicants submit that it is well settled that the enablement requirement does not mean the specification needs to disclose working examples for every species encompassed by the claims, even in an unpredictable art. See *In re Vaeck*, 947 F.2d 488 (Fed. Cir. 1991). Rather, in the biotechnological arts, the disclosure must adequately guide one of skill in the art to determine, without undue experimentation, which species encompassed by the claimed genus possess the disclosed utility.

In the present case, Applicants' claims recite the final step of selecting transformed cells or tissue that are insensitive to galactose toxicity. Therefore, if any enzymatic steps located downstream from the transformed enzyme were to negate selection, the transformed cells will not be insensitive to the galactose toxicity when galactose is added. Therefore, these cells will not be selected because they will not survive. Thus, the claims exclude from their scope transformed cells with rate-limiting steps that negate the selective advantage.

For all of the foregoing reasons, Applicants respectfully submit that the Examiner has not met the burden necessary to rebut the presumption of enablement with respect to the transformation and selection of other plant cells or tissues, at least for this reason. MPEP 2164.04.

The Examiner also asserts that the claims are not enabled for the transformation of plant cells or tissue using any other polynucleotide encoding an enzyme that enhances conversion of galactose to UDP-glucose, beyond *galT*.

Applicants respectfully disagree. Applicants' claims are limited to the transformation of plant cells or tissue, and are further limited to transforming the cells with one or more polynucleotide molecules encoding one or more enzymes useful in the conversion of galactose to UDP-glucose. As stated above, the enzymes recited in the claims are from the well-characterized galactose metabolic pathway. Applicants submit that it would not be an undue burden to one of skill in the art to adapt Applicants' techniques to the limited number of well known enzymes in this pathway. Indeed, the Examiner has not provided any evidence to the contrary. In view of the well-characterized enzymatic pathway, the disclosure and working examples, and lack of any evidence to the contrary, the Examiner has not rebutted the

presumption of enablement with respect to the transformation of cells with polynucleotides other than *galT*.

Due to the foregoing reasons, Applicants respectfully submit that the claims are clearly enabled for their full scope. Withdrawal of the enablement rejection is requested.

Indefiniteness

Claims 81-86 stand rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the invention. The Examiner stated that these dependent claims fail to further limit claim 74.

Applicants respectfully traverse this rejection. Applicants have amended claims 81-83 to clarify that these claims narrow claim 74 by reciting the specific manner of exposing the cells or tissue to galactose. Withdrawal of this rejection is respectfully requested.

Claim 75 stands rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for depending from itself. Claim 75, as amended, now depends from claim 74. Withdrawal of this rejection is requested.

Anticipation

Claims 74-75, 79-86, and 88-89 stand rejected under 35 U.S.C. § 102(b) as anticipated by Mollet et al. The Examiner asserts that Mollet teaches the transformation of galactose sensitive mutants of *E. coli* with genes from *Lactobacillus helveticus* coding for *galK* and *galT*, and growth and selection for transformed cells that overcome the galactose toxicity.

Applicants respectfully traverse this rejection. Claims 74-89 of the present invention recite a process for selecting transformed cells or tissue comprising transforming plant cells or tissue. As the Examiner acknowledges, however, Mollet et al. teach the transformation of *E. coli*. Since *E. coli* strains are bacteria, and not plants or tissue, Applicants submit that Mollet et

al. do not teach all elements of the rejected claims. Withdrawal of this rejection is respectfully requested.

Applicants further note that the present claims recite that galactose is toxic to nontransformed cells or tissue. Applicants submit that Mollet et al. do not teach galactose as toxic to nontransformed cells, but merely suggest that cells lacking a galactose metabolizing enzyme are unable to grow when provided only with minimal galactose-containing media. In other words, the bacterial cells lacking a galactose metabolizing enzyme are unable to utilize galactose as a nutrient; however, this does not mean that the galactose is toxic to these cells. Accordingly, claims 74-75, 79-86, and 88-89 cannot be anticipated by Mollet et al.

Obviousness

Claims 74-75 and 79-90 stand rejected under 35 U.S.C. § 103 as unpatentable over Bojsen in view of Mollet. The Examiner cites Bojsen as teaching transformation of cells from tobacco and sugar beet with cDNA encoding mannose and xylose metabolizing enzymes selected for on mannose or xylose containing medium. The Examiner asserts it would be obvious to modify the transformation of plants with polynucleotides encoding mannose/xylose enzymes and selection on mannose/xylose as disclosed in Bojsen, with transformation with polynucleotides encoding the galactose enzymes and selection on galactose as disclosed in bacteria by Mollet.

Applicants respectfully traverse this rejection. In order to establish a prima facie case of obviousness, the Examiner must show a) that the references disclose all of the elements of the invention, b) that there is a motivation to combine the references to modify the teaching of the reference to obtain Applicants' claimed invention, and c) a reasonable expectation of success.

Applicants submit that the Examiner has not established a prima facie case of obviousness at least because the references provide no teaching or suggestion that would motivate one of skill in the art to replace selection of cells using the mannose/xylose metabolic pathways in plants, as taught by Bojsen et al., with selection using the galactose metabolic pathway in bacteria as taught by Mollet et al. Specifically, neither Bojsen et al. nor Mollet et al. teach or suggest selection of plant cells transformed with nucleotides encoding enzymes that convert galactose to UDP-glucose via insensitivity to galactose toxicity. Rather, Bojsen et al. merely teach that the transformation of plants with polynucleotides encoding enzymes in the mannose/xylose pathways will result in insensitivity to mannose/xylose toxicity. Mollet et el. merely teach that replacing a deleted, endogenous galactose metabolizing enzyme of one bacteria, *E. coli*, with a corresponding galactose metabolizing enzyme from a different bacteria, *Lactobacillus helveticus,* can restore the ability of the *E. coli* to metabolize, and grow on, galactose. In fact, Mollet et al. do not teach selection using galactose as an alternative to traditional selection using antibiotic resistance. Each of the galactose plates in Mollet et al. comprise antibiotics for classical selection of transformed cells. Thus, the successfully transformed cells in Mollet et al. are selectable due to the presence of an antibiotic resistance gene. The cells which have been successfully complemented are identified by their ability to metabolize galactose as the sole carbon source from minimal media, while the nontransformed cells are unable to metabolize the galactose. Mollet et al. nowhere disclose, however, that galactose is toxic to cells not transformed with the enzyme useful in the conversion of galactose to UDP-glucose, while transformed cells are insensitive to the galactose, as is recited by the present claims. Therefore, Mollet et al. do not provide any teaching or suggestion that transforming an *E. coli* cell, let alone the plant cells or tissue of the present claims, with a

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galactose metabolizing enzyme can render the cell insensitive to galactose toxicity. Applicants respectfully submit, therefore, that one of skill in the art would find no motivation from reading Mollet et al. to replace selection of plant cells with the mannose/xylose metabolic pathways as taught by Bojsen et al., with selection using the entirely different galactose metabolic pathway in bacteria as taught by Mollet et al., to create transformed cells insensitive to galactose toxicity, nor would there be any reasonable expectation of success in doing so. Applicants submit, therefore, that claims 74-75 and 78-89 are patentable over Bojsen et al. in view of Mollet et al. at least for this reason. Withdrawal of this rejection is requested.

Conclusion

Applicants submit that all claims are in condition for allowance, and notice of such allowance is earnestly solicited. The Examiner is invited to telephone the undersigned attorney for clarification of any of the amendments and remarks or to otherwise speed prosecution of this application.

Respectfully submitted,

MERCHANT & GOULD, P.C.
P.O. Box 2903
Minneapolis, Minnesota 55402-0903
(612) 332-5300

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By: Garen Gotfredson

Garen J. Gotfredson
Reg. No. 44,722